Childhood asthma exacerbations and the Arg16 β2-receptor polymorphism

Turner, S.; Francis, Ben; Vijverberg, Susanne; Pino-Yanes, Maria; Maitland-van der Zee, Anke H.; Basu, Kaninika

Published in:
Journal of Allergy and Clinical Immunology

DOI:
10.1016/j.jaci.2015.10.045

Publication date:
2016

Childhood asthma exacerbations and the Arg-16 beta2 receptor polymorphism: a meta-analysis stratified by treatment

Steve Turner MD[1], Ben Francis PhD[2], Susanne Vijverberg PhD[3], Maria Pino-Yanes PhD[4,5], Anke H Maitland-van der Zee PhD[3], Kaninika Basu MD[6], Lauren Bignell MBBS[6], Somnath Mukhopadhyay MD[6,7], Roger Tavendale PhD[7], Colin Palmer PhD[7], Daniel Hawcutt PhD[8], Munir Pirmohamed PhD[9], Esteban G Burchard MD MPH[10,11] and Brian Lipworth MD[12] on behalf of the Pharmacogenomics in Childhood Asthma consortium

[1]Child Health, University of Aberdeen

[2] Department of Biostatistics, University of Liverpool, Liverpool

[3]Division of Pharmacoepidemiology and Clinical Pharmacology, University of Utrecht

[4]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain

[5]Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain

[6]Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton, United Kingdom.

[7]Population Pharmacogenetics Group, University of Dundee

[8]Department of Women's and Children's Health, Institute of Translational Medicine, University of Liverpool, Liverpool

[9] Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool

[10] Department of Bioengineering and Therapeutic Sciences and Medicine, University of California, San Francisco, USA

[11]Center for Genes, Environment and Health, University of California, San Francisco, USA

[12] Asthma and allergy Research Group, University of Dundee

Contact details: Dr S Turner, Child Health, Royal Aberdeen Children's Hospital, Aberdeen, AB25 2ZG tel +44 1224 438475, fax +44 1224 438469, s.w.turner@abdn.ac.uk

Funding sources. There were no funds for the present analysis and the individual cohorts were funded as follows:

BREATHE was funded by Scottish Enterprise Tayside, the Gannochy Trust, the Perth and Kinross City Council and Brighton and Sussex Medical School.

GALA II. The contribution from GALA II to this work was supported by grants from National Institutes of Health to E.G.B.: the National Heart, Lung and Blood Institute (HL088133, HL078885, HL004464, HL104608 and HL117004); the National Institute of Environmental Health Sciences (ES015794); the National Institute on Minority Health and Health Disparities (MD006902); the National Institute of General Medical Sciences (GM007546). E.G.B was also funded by the American Asthma Foundation, the RWJF Amos Medical Faculty Development Award, the Sandler Foundation and the Flight Attendant Medical Research Institute. MPY was supported by a postdoctoral fellowship from Fundacion Ramon Areces (www.fundacionareces.es).

PACMAN was funded by a strategic alliance between GSK and Utrecht Institute for Pharmaceutical Sciences.

PAGES was funded by the Chief Scientist Officer for Scotland.

PASS was funded by the UK department of health through the "NHS Chair of Pharmacogenomics

None of the authors has any financial relationship with a biotechnology and/or pharmaceutical manufacturer that has an interest in the subject matter or materials discussed in the submitted manuscript.

Running head: ADRB2 and childhood asthma exacerbations

Word count for main body of manuscript: 2793

ABSTRACT

Background. The Gly-to-Arg substitution at the 16 position (rs1042713) in the beta 2 adrenoceptor (*ADRB2*) gene is associated with enhanced down-regulation and uncoupling of beta-2 receptors.

Objectives. To undertake a meta-analysis to test the hypothesis that there is an interaction between the A allele of rs1042713 (Arg16 amino acid) and long acting beta agonist (LABA) exposure for asthma exacerbations in children.

Methods. Children with diagnosed asthma were recruited in five populations (BREATHE, GALA II, PACMAN, PAGES and PASS). A history of recent exacerbation and asthma treatment were determined from questionnaire data. DNA was extracted and the Gly16Arg genotype determined.

Results. Data from 4226 children of white Northern European and Latino origin were analysed and the odds ratio for exacerbation increased by 1.52 [1.17, 1.99] p=0.0021 for each copy of the A allele among the 637 children treated with inhaled corticosteroids (ICS) plus LABA but not for treatment with ICS alone (n=1758), nor ICS plus leukotriene receptor antagonist (LTRA, n=354) or ICS plus LABA plus LTRA (n=569).

Conclusions. The use of LABA as "add-on controller", but not LTRA, is associated with increased risk of asthma exacerbations in children carrying one or two A alleles at rs1042713. Prospective genotype stratified clinical trials are now required to explore the potential role of rs1042713 genotyping for personalised asthma therapy in children.

Key words: Adrenergic receptors; Asthma; Child; Disease exacerbation; Therapeutics.

79 KEY MESSAGE

80 Clinical trials are required to determine whether treatment stratified by rs1042713 will
81 reduce asthma exacerbation risk in children with one or two A alleles.

82

CAPSULE SUMMARY

This meta-analysis finds increased asthma exacerbation risk for children who carry ≥ 1 A allele of rs1042713, but only in the context of treatment with inhaled corticosteroids and long acting beta agonist.

88 **ABBREVIATIONS**

89 ADRB2 Beta 2 Adrenoceptor

90 LABA Long Acting Beta Agonists

91 LTRA Leukotriene Receptor Antagonist

92 ICS Inhaled Corticosteroids

93 MAF Minor Allele Frequency

94 PAGES Paediatric Asthma Gene Environment Study

95 PASS Pharmaco genetics of Adrenal Suppression with inhaled Steroid study

96 SABA Short Acting Beta Agonists

97 SNP Single Nucleotide Polymorphism

98

INTRODUCTION

Asthma is a common condition in children where there is heterogeneity in response to treatment with inhaled corticosteroids (ICS), long acting beta agonists (LABA) and leukotriene receptor antagonists (LTRA) [1, 2]. Some of this heterogeneity may reflect genetic variations within the population, and variants in the gene coding for the beta 2 adrenoceptor (*ADRB2*) have been associated with increased risk for symptoms [3-5]. Of particular interest is the single nucleotide polymorphism (SNP) rs1042713, a Gly to Arg amino acid substitution at the position 16 of the *ADRB2* gene, which has been associated with differences in pulmonary function responsiveness to short acting beta agonists in children [6-9] and the underlying mechanism of enhanced down-regulation and uncoupling of beta-2 receptors is thought to reflect an altered response to short and long acting beta agonists (SABA and LABA).

Although the SNP rs1042713 appears to alter physiological and clinical response to SABA and LABA in paediatric populations, the clinical relevance of this association remains unclear. In two clinical trials there was no evidence for an association between the A allele of rs1042713 (Arg16 amino acid) and increased symptom score [1, 7]. There is inconsistent evidence from observational studies that this SNP may be relevant to exacerbations. In children, the homozygous G/G genotype of rs1042713 has been linked with increased risk for hospitalisation[10], reduced bronchodilator response to short acting beta agonists[9], prolonged stay in hospital [11] and intensive care unit stay [12] after presentation with acute asthma, whilst the heterozygous genotype of rs1042713 has been linked with increased risk for intubation for acute asthma [13]. Two other groups have observed associations between A/A genotype of rs1042713 and increased exacerbations among those treated with LABA [3, 4]

but this was not confirmed in a third population[14]. These studies have also observed increased exacerbation risk [3] and poorer asthma control [4] among those children homozygous for A/A for the SNP rs1042713 in receipt of ICS (but not LABA). In one study [3], there was evidence that concomitant LTRA treatment might negate any increased risk for exacerbation associated with LABA treatment, while those children who are homozygous for Arg16 had better asthma outcomes when treated with LTRA rather than LABA in addition to to ICS [15]. Prospective studies undertaken in adult populations have found no evidence for LABA treatment being associated with adverse outcomes when added to ICS[16-18]

To better understand the interactions between the SNP rs1042713 of *ADRB2* and asthma treatment, we undertook a meta-analysis of results from five previously described populations [19]. Our hypothesis was that there is an interaction between the A allele of rs1042713 (Arg16 amino acid) and treatment with LABA, but not LTRA, for asthma exacerbation risk and that this risk might be further increased by exposure to daily SABA.

METHODS

Study design. Children with asthma were recruited to five cross-sectional studies (BREATHE, GALA II, PAGES, PACMAN and PASS). The BREATHE and PAGES populations were recruited from primary and secondary care in Scotland, PACMAN was recruited from children attending community pharmacists in Netherlands, GALA II recruited children in USA and Puerto Rico who had four Latino grandparents and PASS recruited children with asthma who had adrenal suppression testing in 25 hospitals across the UK. Further details of the study population's recruitment are presented in the Online Repository. DNA was extracted from saliva or blood and the genotypes for rs1042713 determined. The primary outcome was asthma exacerbation (with reference to six months in BREATHE, PAGES and PASS and 12 months in GALA II and PACMAN). Asthma treatment was categorised thus: (i) as required (prn) SABA but no preventer treatment (ii) ICS monotherapy plus prn SABA (iii) ICS plus prn SABA and LABA (iv) ICS plus prn SABA and LTRA and (v) ICS plus prn SABA, LABA and LTRA. As previously [5], as required SABA was categorised as at least once daily or less frequently. Approval was obtained from medical research ethics committees from each institute prior to recruitment. All participants gave verbal assent and parents or participants gave written consent as appropriate.

Definitions of exacerbation. For BREATHE and PAGES the definition of exacerbation was at least one of the following in the previous six months in the context of asthma symptoms: hospital admission, course of oral steroids or absence from school. For GALA, an exacerbation was defined as at least one of the following during the previous 12 months: oral corticosteroid rescue treatment, hospitalisation or need to seek emergency asthma care. For PACMAN, an exacerbation was defined as an asthma-related visit to emergency

department and/or prescribed a course of oral steroids in the past twelve months. The definition of exacerbation for PASS was at least one course of rescue oral steroids in the previous six months.

DNA collection, extraction and analysis. For BREATHE, PACMAN and PAGES, saliva was collected in commercially available pots (Oragene, DNA Genotech, Ontario, Canada) DNA was prepared using the Qiagen Dneasy 96 kit, and genotypes were determined in the Dundee laboratory using Taqman based allelic discrimination assays on an ABI 7700 sequence detection system, as described previously[3]. For GALA II, DNA was extracted from whole blood and the Axiom® LAT1 array (World Array 4, Affymetrix, Santa Clara, CA) was used to determine genome wide-genotype data as described elsewhere[20]. For PASS, the Illumina Human OmniExpressExome-8 v1.0 chip (Illumina, San Diego, CA) was used for genotyping.

Statistical analysis. The primary outcome was recent exacerbation and this was related to genotype in logistic models. An additive model [3] was used, i.e. a gene/dosage effect for the A allele [Arg16 aminoacid] which adjusted for confounders (i.e. gender, age, second hand smoke exposure [3]). Each population was stratified by treatment and risk for exacerbation per genotype was calculated in each treatment group. Daily SABA use was recorded for BREATHE, PACMAN and PAGES and here an interaction as sought for SABA treatment x genotype. Regression analyses in GALA II included the same covariates as in the other studies, but additionally we included estimates of global African and Native American genetic ancestry to avoid confusion due to population stratification. Standard statistical software was used (SPSS version 22.0.0.1). The meta-analysis of data from the five populations was performed using a fixed-effect (inverse variance–weighted) model, where

183 the effect size estimates, *β* coefficients, are weighted by their estimated standard errors
184 using GWAMA software[21]. We estimated the power of the study to detect the associations
185 with exacerbations following the methodology of Purcell *et al* [22]. Our power calculations
186 provide the maximal power we could obtain from the meta-analysis of the cohorts at the
187 significance level of 5%. The odd ratios of 1.2, 1.5 and 3 were selected based on initial
188 results from the BREATHE trial. With the exception of the ICS+LTRA treatment group, all
189 strata were sufficiently powered to detect an odds ratio of 1.5 or above (See table I in the
190 Online Repository) . Forest plots were generated with the package *rmeta* for R. A p value of
191 <0.05 was assumed to be significant.

192

RESULTS

Study subjects

Genotype, treatment and exacerbation data were available in 4226 children including 1210 from BREATHE, 1171 from GALA II, 760 from PACMAN, 695 from PAGES and 390 from PASS, table I. The Gly16Arg polymorphism was in HWE for all cohorts with the exception of BREATHE (exact test p=0.012) considered as a whole, but it was in HWE in the group of children that did not have exacerbations (p=0.624). The minor allele frequency (MAF) for GALA II was higher when compared to the three UK cohorts (0.45 vs 0.37) $p=1x10^{-10}$, and intermediate for the PACMAN population. Regardless of treatment, across the five populations the additive model found an increased risk for exacerbation for each copy of the A allele amounting to 1.11 [1.01, 1.22] p=0.035, n=4226 (See table II in the Online Repository).

Risk of exacerbation across maintenance treatment groups

The odds ratio for exacerbation was 1.52 [1.17, 1.99] for each copy of the A allele among the 637 children treated with ICS plus LABA, table II. The risk for exacerbation was not increased among other treatment groups, table II. Table III presents the proportion of children with exacerbations stratified by population, treatment and genotype. The analysis for children treated with ICS plus LABA had >90% power to detect an association with increased risk for exacerbation at the significance level of 5% (Table I in the Online Repository). An analysis of local African ancestry at the Gly16Arg locus was undertaken in the GALA II population to examine if the number of chromosomes indicative of African ancestry at this locus was associated with increased exacerbations. There was no

association of local African ancestry with exacerbations in GALA II in the overall population (OR=1.17, 95% CI: 0.89-1.53, *p*=0.270) or in the group of patients treated with ICS plus LABA (OR=1.78, 95% CI: 0.58-5.49, *p*=0.316).

Risk of exacerbation in relation to SABA use

Among the 822 children in receipt of daily SABA (including 56 who were not on ICS, LABA or LTRA) there was no evidence of increased risk in the additive model (OR 1.01 [0.79, 1.31], see table III in the Online Repository)). Among those children in receipt of ICS plus LABA there was no evidence of any additional increased risk in relation to each A allele for exacerbations among those taking daily SABA (see table IV in the Online Repository).

Asthma control scores and Arg16 homozygous genotype

The risk for poorly controlled asthma (as evidence by the asthma control questionnaire 6 score >1.5) was increased among A/A homozygotes prescribed ICS only within the cohort PACMAN (OR 2.15) [(4)]. Within the PAGES population 63% (282/446) were poorly controlled (as evidenced by Children's Asthma Control Test score <20) and there was no increase in risk for poor control for A/A homozygotes among any treatment groups.

DISCUSSION

Genetic epidemiology is complicated by inconsistent findings between populations. Therefore replication of findings across different populations is crucial to generalising results [23]. Associations between SNP rs1042713 and LABA and SABA treatment have been previously reported in evaluations of the first 546 children recruited to BREATHE[3] and the first 597 recruited to PACMAN[4] (data from 1210 and 760 included in the present report respectively). However, the results of other studies in adults were in apparent conflict with the above observations. This meant that, prior to this study, the important clinical question of whether or not there is a need to progress to further randomised controlled trials assessing benefit with testing for SNP rs1042713 in the clinical setting had not been resolved. This study combined data from five cohorts of children with asthma from white European and Hispanic/Latino populations to explore interactions between exposures to different asthma medications and the SNP rs1042713 for risk of asthma exacerbation. We analysed data from 4226 children and draw three conclusions. First, among children exposed to ICS plus LABA as dual combination therapy there was a 52% increased risk for exacerbation for each copy of the A allele. Second, the interaction between the A allele and exposure to LABA was not present when LTRA treatment was also co-prescribed as triple therapy. Third, there was no evidence that daily SABA usage in addition to ICS plus LABA was associated with any increased further risk for exacerbation among children carrying at least one A allele. The combined incidence of A/G heterozygous and A/A homozygous genotype is approximately 60% and these observations implicate the SNP rs1042713 as an important factor in the well-recognised heterogeneity of treatment response in children with asthma[1, 2]. This study has established the need for further prospective clinical trials where treatment is stratified by genotype to

move these observations into clinical practice in order to evaluate a more personalised approach to treatment of children with poorly controlled asthma despite treatment with inhaled corticosteroids.

We observed heterogeneity between populations for the relationship between SNP rs1042713 and treatment with ICS plus LABA and risk for exacerbation with the risk being highest in GALA II and lowest in PASS. Although this study was not designed to explain the variability between populations, there was no obvious association between the effect size for exacerbation risk associated with A allele and characteristics of the five populations; for example the children in GALA II and PASS were comparable in terms of age, sex distribution and exacerbation rate. More children in PASS were in receipt of ICS plus LABA compared to GALA II but the hypothesis that exacerbation risk attributable to the A allele is lower for populations where LABA treatment is more prevalent is not supported by observations in the PACMAN and PAGES populations where 19% in each received LABA but the exacerbation risk associated with ICS plus LABA was 2.54 and 1.29 respectively. The heterogeneity between populations, and within populations [1, 2], might give potential insight into the pharmacogenetic mechanism(s) but also highlights the need for stratified treatment in childhood asthma.

The minor allele frequency was substantially higher for children in the GALA II population compared to the three UK populations and, as suggested by previous work [18, 24], we explored the possibility that the increased exacerbation rate associated with the Arg16 allele in LABA-treated GALA II subjects reflected the African ancestry associated with this allele. In our adjustment for measures of ancestry for the analyses of the Gly16Arg locus within the GALA II population we did not find significant evidence that African ancestry was

277 relevant to the positive correlation between minor allele frequency and prevalence of
278 exacerbation, however our analysis was underpowered and the two-fold increase in risk
279 which was detected might have been significant had our sample size been larger. Our study
280 was not designed to explore how ethnic differences might be relevant to the
281 pharmacogenetics or treatment response to LABA and unfortunately there were insufficient
282 numbers of children with African ancestry in the cohorts other than GALA II to further
283 explore this intriguing hypothesis which merits focussed research in future.

284 The pharmacogenetics of LABA and SABA are notable for the contrasting effects seen for the
285 Gly16Arg locus on acute versus chronic SABA. There has been considerable consistency in
286 the observed effects of Gly16Arg on acute SABA response with many studies showing a
287 similar direction of effect on bronchodilation (favouring Arg16)[6, 7, 11, 25, 26]. A seemingly
288 opposite effect (favouring Gly16) was seen for chronic SABA exposure and lung function and
289 asthma control in the Beta Agonists in Mild Asthma (BAGS) [27] and Beta-Adrenergic
290 Response by Genotype (BARGE) [28] studies and another study by Taylor et al [29]. The focus
291 of the present study was LABA therapy but we found no evidence for either daily SABA use
292 or the combination of daily SABA plus LABA being linked with increased exacerbation risk for
293 children carrying Arg16 allele. One interpretation of our findings is that the LABA caused
294 effective adrenoceptor blockade and the “adverse” effect of LABA treatment had subsumed
295 any “benefit” of acute SABA treatment for individuals carrying an Arg16 allele.

296

297 Although we find evidence here for response to LABA therapy to be modified by the
298 Gly16Arg locus in children, for adults this locus appears to have no effect on response to
299 LABA therapy based on large retrospective analyses and prospective genotype-stratified

clinical trials by Bleecker [16] and the LARGE trial [18]. There is evidence that the Gly16 allele might be associated with a bronchoprotective effect in association with LABA treatment [18, 30]. The present study was not designed to explore the apparent inconsistency between observations in adults and children but differences in response of children and adults to LABA are well-recognised. The addition of LABA to ICS treatment in adults with poorly controlled asthma is accepted to be superior to alternative treatments [31, 32] but in children the evidence is that LABA are no more effective that addition of LTRA or increase in ICS [1, 33].

Our analysis included a relatively large number of well-characterised children with asthma from five populations and therefore any significant association is likely to be generalizable to other similar populations, perhaps aside from those where there is a higher proportion of individuals of African descent where the A allele is more prevalent. In the literature there are apparently conflicting associations reported between variants of the SNP rs1042713 and response to treatment [3-6, 9, 11] and one explanation for this heterogeneity is that the earlier findings are based on relatively small single populations, i.e. which number less than 1000, and meta-analysis addresses the potential for false positive findings and/or associations which are idiosyncratic for one population. This meta-analysis confirms previously reported increases in the risk for exacerbations among Arg16 homozygotes in receipt of LABA treatment [3, 4]. The present study failed to replicate previously reported associations between homozygous genotype A/A and treatment with ICS alone and increased risk for exacerbation [3] or poor asthma control [4], suggesting the possibility of false positive findings. The magnitude of risk for exacerbation associated with ICS and LABA treatment and A allele reported in first 546 children recruited to the BREATHE cohort [3] is slightly

reduced in the larger population (2.1 versus 1.5) but remains significant across all five populations. The five populations included were heterogeneous for asthma outcomes and the results could be generalised to Western European and Hispanic/Latino populations but, given the potential for different associations between rs1042713 and asthma treatment response between different ethnic groups [26], our results may not be relevant to all populations.

In our analysis we explored the possible additive effect of treatment with daily SABA and LABA for exacerbations, and we have previously reported that either treatment is associated with increased exacerbations for the BREATHE population among Arg/Arg homozygotes[5]. When data were pooled there was no apparent additive effect of SABA on LABA for exacerbation risk for children with one or two Arg alleles. These results should be treated with caution since, even with a relatively large population such as we present here, there were relatively few children with ICS plus LABA and daily SABA exposure and the analysis was probably underpowered and there may be a modest additive effect which we were not able to detect.

The mechanism(s) underlying the association between the Arg16 allele and increased exacerbation in the context of LABA treatment are thought to be mediated by enhanced agonist induced down-regulation and receptor uncoupling, resulting in subsensitivity of response [34, 35]. Our novel finding of no increased risk for exacerbation among A/A homozygotes in receipt of ICS and LABA and LTRA suggests that factors other than *ADRB2* down regulation are active since LABA exposure in this group of children might be expected to down regulate *ADRB2*. It is likely that LTRA merely confer an additional anti-inflammatory effect in individuals exposed to LABA, such that in genetically susceptible

patients it might be seen as a salutary effect by counteracting the response to LABA. Where the LABA effect is negated (i.e. in Arg/Arg) the additive effect of LTRA will be more evident compared to the setting where LABA effect is more pronounced (i.e. Gly/Gly) and the additive effect of LTRA will be less evident. Indeed in one study using AMP challenge as the primary outcome, there was better protection with ICS/LABA/LTRA as triple therapy compared to dual therapy with ICS/LABA, which was also mirrored by effects on exhaled NO and blood eosinophils, suggesting that the apparent counteracting role of LTRA may arise from the additional anti-inflammatory effects of LTRA [(36)].

This study has a number of limitations, which should be considered when these results are interpreted. First, the associations described here do not imply causation but the findings are consistent with the results of a small genotype stratified randomised controlled trial, which found favourable outcomes among A/A homozygotes taking LTRA compared to LABA over a period of 12 months when used as add on therapy to ICS [(15)]. Second, although we are able to be conclusive as to the nature of the relationship between exposure to LABA and A/A homozygous genotype and exacerbation, we cannot exclude the possibility that there may be a small additive relationship between SABA and LABA for exacerbation. The relationship between LABA, SABA and exacerbations will always be a challenge to study since frequent SABA treatment is an indication for LABA therapy but our findings suggest that the magnitude of association with LABA is greater than SABA, which perhaps is not surprising given the potential impact more prolonged receptor occupancy conferred by LABA than SABA, especially in genetically susceptible individuals. Third, more detailed genotyping of the ADRB2 locus ~~a genome wide association study (GWAS)~~ or haplotype analysis might have yielded additional insight into the relationship between genetic

variations of the *ADRB2* but neither GWAS or haplotype data were available for all cohorts and we focussed on the SNP rs1042713 since there is a large literature which indicates that this is associated with outcomes for asthma treatment. A fourth limitation is that we have assumed that treatment has been assigned based on the same criteria and it is possible that in some cohorts, children with more severe asthma and at increased risk for exacerbations might not have received LABA treatment but this would tend to underestimate the effect of the interaction between LABA treatment and the SNP rs1042713 for exacerbations. A fifth limitation is that rare variants were not genotyped which could also have an impact on adverse events during LABA therapy; however, these cohorts were not powered for a rare variant analysis and the rare variants identified by Ortega *et al* [38] occurred in the background of Gly16, not Arg16. Finally, the polymorphism Gly16Arg allele frequency was not in Hardy Weinberg Equilibrium for the whole BREATHE cohort but the consistency of the results across the cohorts suggests that the deviation within this single cohort did not substantially influence the overall results. Furthermore, Gly16Arg is an inconsistently replicated and, at best, weak locus for asthma severity so it does not seem plausible that selection for exacerbation (the primary outcome and linked to asthma severity) in this study is the cause for the deviation from HWE seen in the BREATHE cohort. Since Gly16Arg is a better established pharmacogenetic locus, a more plausible outcome which would influence deviations from HWE is exacerbations or symptoms despite frequent SABA use or LABA use, but this represents a small percentage of the BREATHE cohort (18% and 11%, respectively).

In conclusion, children with asthma on ICS plus LABA were at 52% increased risk for exacerbation in the previous 6-12 months for each A allele (Arg16 amino acid) compared to G/G homozygotes (Gly16/Gly16). Knowing that there are 1 million children in the UK with

asthma and 10% of these are prescribed LABA [39~~38~~] and 60% of these carry at least one Arg16 allele, there are approximately 60,000 children in the UK today, and approximately 25,000 in the Netherlands, who might be at risk from the morbidity of exacerbation which is preventable by treatment with either no LABA or perhaps with the addition of LTRA if genotyping of rs1042713 could be made available at the point of prescribing. Put another way, and assuming that at least one third of the UK national healthcare costs attributable to childhood asthma are due to urgent care[40, 41~~39, 40~~], stratified treatment might reduce the annual direct costs to the UK National Health Service for the management of childhood asthma exacerbations by 10% (i.e. a 50% reduction in 60% of the population). Whilst there are no recent published costs for the management of childhood asthma, costs in the US have been estimated at $791per child per annum in 2005 [41~~40~~] and to total £150 million in the UK in 1997 [42~~41~~]. It is thus likely that stratified treatment in childhood asthma will save tens of millions of pounds in direct healthcare costs. Not all asthmatics who inherit an A allele will experience increased morbidity with LABA treatment and this might be explained by rare variants, perhaps at the same locus [38~~42~~], pathway related variation[43], epigenetic mechanisms or treatment adherence.

REFERENCES

1. Lemanske RF Jr. Mauger DT. Sorkness CA. Jackson DJ. Boehmer SJ. Martinez FD. Strunk RC. Szefler SJ. Zeiger RS. Bacharier LB. Covar RA. Guilbert TW. Larsen G. Morgan WJ. Moss MH. Spahn JD. Taussig LM. Childhood Asthma Research and Education (CARE) Network of the National Heart, Lung,and Blood Institute. Step-up therapy for children with uncontrolled asthma receiving inhaled corticosteroids. N Engl J Med 2010;362:975-85.

2. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC*, et al*. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-42.

3. Palmer CN, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax. 2006;61:940-4.

4. Zuurhout MJ, Vijverberg SJ, Raaijmakers JA, Koenderman L, Postma DS, Koppelman GH*, et al*. Arg16 ADRB2 genotype increases the risk of asthma exacerbation in children with a reported use of long-acting beta2-agonists: Results of the PACMAN cohort. Pharmacogenomics 2013;14:1965-71.

5. Basu K, Palmer CN, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic beta(2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. J Allergy Clin Immunol 2009;124:1188,94.e3.

6. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J Clin Invest 1997;100:3184-8.

7. Silverman EK, Kwiatkowski DJ, Sylvia JS, Lazarus R, Drazen JM, Lange C*, et al*. Family-based association analysis of beta2-adrenergic receptor polymorphisms in the childhood asthma management program. J Allergy Clin Immunol 2003;112:870-6.

8. Turner SW. Genetic predictors of response to therapy in childhood asthma. Mol Diag Ther 2009;13:127-35.

9. Finkelstein Y, Bournissen FG, Hutson JR, Shannon M. Polymorphism of the ADRB2 gene and response to inhaled beta- agonists in children with asthma: A meta-analysis. J Asthma 2009;46:900-5.

10. Turner SW, Khoo SK, Laing IA, Palmer LJ, Gibson NA, Rye P*, et al*. beta2 adrenoceptor Arg16Gly polymorphism, airway responsiveness, lung function and asthma in infants and children. Clin Exp Allergy 2004;34:1043-8.

11. Carroll CL, Stoltz P, Schramm CM, Zucker AR. Beta2-adrenergic receptor polymorphisms affect response to treatment in children with severe asthma exacerbations. Chest 2009;135:1186-92.

12. Carroll CL, Sala KA, Zucker AR, Schramm CM. Beta-adrenergic receptor polymorphisms associated with length of ICU stay in pediatric status asthmaticus. Pediatr Pulmonol 2012;47:233-9.

13. Carroll CL, Sala KA, Zucker AR, Schramm CM. beta2-adrenergic receptor haplotype linked to intubation and mechanical ventilation in children with asthma. J Asthma 2012;49:563-8.

14. Giubergia V, Gravina L, Castanos C, Chertkoff L. Influence of beta(2)-adrenergic receptor polymorphisms on asthma exacerbation in children with severe asthma regularly receiving salmeterol. Ann Allergy Asthma Immunol 2013;110:156-60.

15. Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA*, et al*. Tailored second-line therapy in asthmatic children with the arg(16) genotype. Clin Sci 2013;124:521-8.

16. Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of ADRB2 polymorphisms on response to long acting beta2-agonist therapy: A pharmacogenetic analysis of two randomised studies. Lancet 2007;370:2118-25.

17. Bleecker ER, Nelson HS, Kraft M, Corren J, Meyers DA, Yancey SW*, et al*. Beta2-receptor polymorphisms in patients receiving salmeterol with or without fluticasone propionate. Am J Respir Crit Care Med 2010;181:676-87.

18. Wechsler ME. Kunselman SJ. Chinchilli VM. Bleecker E. Boushey HA. Calhoun WJ. Ameredes BT. Castro M. Craig TJ. Denlinger L. Fahy JV. Jarjour N. Kazani S. Kim S. Kraft M. Lazarus SC. Lemanske RF Jr. Markezich A. Martin RJ. Permaul P. Peters SP. Ramsdell J. Sorkness CA. Sutherland ER. Szefler SJ. Walter MJ. Wasserman SI. Israel E. National Heart,Lung and Blood Institute's Asthma Clinical Research Network. Effect of beta2-adrenergic receptor polymorphism on response to long acting beta2 agonist in asthma

(LARGE trial): A genotype-stratified, randomised, placebo-controlled, crossover trial. Lancet 2009;374:1754-64.

19. Vijverberg SJ, Koster ES, Tavendale R, Leusink M, Koenderman L, Raaijmakers JA*, et al*. ST13 polymorphisms and their effect on exacerbations in steroid-treated asthmatic children and young adults. Clin Exp All 2015;45:1051-9.

20. Galanter JM, Gignoux CR, Torgerson DG, Roth LA, Eng C, Oh SS*, et al*. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: The genes-environments and admixture in Latino Americans study. J Allergy Clin Immunol. 2014;134:295-305.

21. Magi R, Morris AP. GWAMA: Software for genome-wide association meta-analysis. BMC Bioinformatics 2010;11:288.

22. Purcell S, Cherny SS, Sham PC. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149-50.

23. Hall IP, Blakey JD. Genetic association studies in thorax. Thorax 2005;60:357-9.

24. Ortega VE, Meyers DA. Pharmacogenetics: Implications of race and ethnicity on defining genetic profiles for personalized medicine. J Allergy Clin Immunol 2014;133:16-26.

25. Lima JJ, Thomason DB, Mohamed MH, Eberle LV, Self TH, Johnson JA. Impact of genetic polymorphisms of the beta2-adrenergic receptor on albuterol bronchodilator pharmacodynamics. Clin Pharmacol Therap 1999;65:519-25.

26. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J*, et al*. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. Am J Respir Crit Care Med 2005;171:563-70.

27 Israel E, Drazen JM, Liggett SB, Boushey HA, Cherniack RM, Chinchilli VM*, et al*. The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma. Am J Respir Crit Care Med 2000;162:75-80

27. Israel E. Chinchilli VM. Ford JG. Boushey HA. Cherniack R. Craig TJ. Deykin A. Fagan JK. Fahy JV. Fish J. Kraft M. Kunselman SJ. Lazarus SC. Lemanske RF Jr. Liggett SB. Martin RJ. Mitra N. Peters SP. Silverman E. Sorkness CA. Szefler SJ. Wechsler ME. Weiss ST. Drazen JM. National Heart, Lung,and Blood Institute's Asthma Clinical Research Network. Use of regularly scheduled albuterol treatment in asthma: Genotype-stratified, randomised, placebo-controlled cross-over trial. Lancet 2004;364:1505-12.

28. Taylor DR, Drazen JM, Herbison GP, Yandava CN, Hancox RJ, Town GI. Asthma exacerbations during long term beta agonist use: Influence of beta(2) adrenoceptor polymorphism. Thorax 2000;55:762-7.

29. Lee DK, Currie GP, Hall IP, Lima JJ, Lipworth BJ. The arginine-16 beta2-adrenoceptor polymorphism predisposes to bronchoprotective subsensitivity in patients treated with formoterol and salmeterol. Br J Clin Pharmacol 2004;57:68-75.

30. British Thoracic Society/Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma 2014. (accessed 2 June 2015 at https://www.brit-

thoracic.org.uk/document-library/clinical-information/asthma/btssign-asthma-guideline-2014/)

31. Global Initiative for Asthma. Pocket guide for asthma management and prevention. (accessed 2 June 2015 at http://www.ginasthma.org/documents/1)

32. Vaessen-Verberne AA, van den Berg NJ, van Nierop JC, Brackel HJ, Gerrits GP, Hop WC*, et al*. Combination therapy salmeterol/fluticasone versus doubling dose of fluticasone in children with asthma. Am J Respir Crit Care Med 2010;182:1221-7.

33. Ellsworth DL, Coady SA, Chen W, Srinivasan SR, Elkasabany A, Gustat J*, et al*. Influence of the beta2-adrenergic receptor Arg16Gly polymorphism on longitudinal changes in obesity from childhood through young adulthood in a biracial cohort: The Bogalusa heart study. Int J Obesity Rel Metabol Dis 2002;26:928-37.

34. Lipworth B. Beta-adrenoceptor genotype and bronchoprotective subsensitivity with long-acting beta-agonists in asthma. Am J Respir Crit Care Med 2013;188:1386-7.

35. Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of beta 2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. Am J Respir Cell Mol Biol 1995;13:25-33.

36. Currie GP, Lee DK, Haggart K, Bates CE, Lipworth BJ. Effects of montelukast on surrogate inflammatory markers in corticosteroid-treated patients with asthma. Am J Respir Crit Care Med 2003;167:1232-8.

37. Ambrose HJ, Lawrance RM, Cresswell CJ, Goldman M, Meyers DA, Bleecker ER. Effect of beta2-adrenergic receptor gene (ADRB2) 3' untranslated region polymorphisms on inhaled corticosteroid/long-acting beta2-adrenergic agonist response. Respir Res 2012;13:37.

38. Ortega VE, Hawkins GA, Moore WC, Hastie AT, Ampleford EJ, Busse WW, *et al*. Effect of rare variants in ADRB2 on risk of severe exacerbations and symptom control during longacting beta agonist treatment in a multiethnic asthma population: A genetic study. Lancet Respir Med 2014;2:204-13.

3938. Turner S, Thomas M, von Ziegenweidt J, Price D. Prescribing trends in asthma: A longitudinal observational study. Arch Dis Child 2009;94:16-22.

4039. Lozano P, Fishman P, VonKorff M, Hecht J. Health care utilization and cost among children with asthma who were enrolled in a health maintenance organization. Pediatr1997;99:757-64.

4140. Wang LY, Zhong Y, Wheeler L. Direct and indirect costs of asthma in school-age children. Prevent Chronic Dis 2005;2:A11.

4241. Lenney W. The burden of pediatric asthma. Pediatr Pulmonol 1997;15:13-6..

~~42. Ortega VE, Hawkins GA, Moore WC, Hastie AT, Ampleford EJ, Busse WW, *et al*. Effect of rare variants in ADRB2 on risk of severe exacerbations and symptom control during longacting beta agonist treatment in a multiethnic asthma population: A genetic study. Lancet Respir Med 2014;2:204-13.~~

43. Kim SH, Ye YM, Lee HY, Sin HJ, Park HS. Combined pharmacogenetic effect of ADCY9 and ADRB2 gene polymorphisms on the bronchodilator response to inhaled combination therapy. J Clin Pharm Therap 2011;36:399-405.

Table I. Comparison of details of children in each of the five study populations. *Dutch, Moroccan and Turkish ethnicities were considered Caucasian in PACMAN. †ethnicity data were not available for all participants in PAGES. ‡daily short acting beta agonist status was not determined in GALA II and PASS.¶ minor allele frequency = frequency of minor allele homozygous genotype + (frequency of heterozygous genotype)/2

		BREATHE n=1210	GALA II n=1171	PACMAN n=760	PAGES n=695	PASS n=390
	% Male (n)	60% (725)	58% (676)	63% (478)	57% (399)	56% (172)
	% Exposed to tobacco smoke at home (n)	35% (424)	21% (242)	14% (108)	21% (146)	36% (108)
	Mean age, (SD) years	9.7 (3.8)	11.9 (2.7)	8.7 (2.3)	9.8 (3.7)	11.1 (4.0)
	Recent exacerbation	44% (536)	65% (763)	10% (76)	47% (323)	75% (295)
Ethicity	% Caucasian	No data	0%	90%* (681/753)	93%† (358/384)	99% (388/390)
	% Hispanic		100%	0.4% (3/753)	0%	0%
	% African		0%	1% (8/753)	0%	0%
	% Other (including mixed)		0%	8.6% (61/753)	7% (26/384)	1% (2/390)
	Minor allele frequency¶	0.37	0.45	0.41	0.37	0.38
	A/A (Arg/Arg) (n)	15% (175)	20% (234)	15% (115)	14% (96)	16% (61)

rs1042713 genotype	A/G (Arg/Gly) (n)	43% (515)	49% (579)	51% (388)	46% (321)	43% (169)
	G/G (Gly/Gly) (n)	43% (520)	31% (358)	34% (257)	40% (278)	41% (160)
Treatment group	% Short acting beta agonist alone (n)	18% (218)	42% (490)	10% (73)	7% (51)	0%
	% Inhaled corticosteroid, ICS, (n)	58% (698)	24% (283)	63% (476)	40% (273)	7% (28)
	% ICS plus long acting beta agonist, LABA, (n)	11% (138)	10% (122)	19% (147)	19% (134)	33% (96)
	%ICS plus leukotriene receptor antagonist, LTRA, (n	5% (65)	15% (177)	3% (23)	9% (65)	8% (24)
	% ICS plus LABA plus LTRA, (n)	8% (91)	9% (99)	5% (41)	24% (169)	59% (230)
% with daily short acting beta agonist dosing, (n)		21% (250)	‡	49% (364)	30% (209)	‡

Table II. Odds ratio for exacerbation per copy of the A allele (Arg16 amino acid). Results are from logistic regression models which adjusted for sex, age and exposure to second hand smoke at home. SABA=short acting beta agonist. ICS =inhaled corticosteroids. LABA= long acting beta agonist. LTRA=leukotriene receptor antagonist. *There were no children in PASS on SABA alone.

Treatment group	Odds ratio [95% confidence interval] for exacerbation per A allele (referenced to none)						
	BREATHE	GALA II	PACMAN	PAGES	PASS	Results from all cohorts combined	Results for all cohorts except GALA II
SABA alone	0.87 [0.54, 1.40] (n=218)	1.08 [0.81,1.43] (n=490)	1.07 [0.06, 20.2] (n=73)	0.71 [0.18, 2.80] (n=51)	*	1.01 [0.79, 1.28] (n=832) p=0.95	0.85 [0.55, 1.33] (n=342) p=0.49
ICS alone	1.15 [0.92, 1.43] (n=698)	1.12 [0.78,1.62] (n=283)	0.83 [0.53, 1.31] (n=476)	1.17 [0.80, 1.71] (n=273)	4.81 [0.79, 29.33] (n=28)	1.11 [0.95, 1.31] (n=1758) p=0.18	1.11 [0.94, 1.33] (n=1475) p=0.22
ICS+LABA	1.52 [0.92, 2.50] (n=138)	2.07 [1.03, 4.16] (n=122)	2.54 [1.06, 6.06] (n=147)	1.29 [0.76, 2.19] (n=134)	1.21 [0.68, 2.14] (n=96)	1.52 [1.17, 1.99] (n=637) p=0.0021	1.44 [1.08, 1.93] (n=515) p=0.01
ICS+LTRA	1.86 [0.85, 4.08] (n=65)	1.26 [0.79, 2.02] (n=177)	2.10 [0.43, 10.2] (n=23)	0.69 [0.34, 1.39] (n=65)	0.31 [0.08, 1.18] (n=24)	1.11 [0.80, 1.55] (n=354) p=0.52	0.98 [0.61, 1.56] (n=177) p=0.93
ICS+LABA+LTRA	1.03 [0.54,1.96] (n=91)	0.93 [0.36,2.39] (n=99)	0.23 [0.04,1.46] (n=41)	0.87 [0.52, 1.45] (n=169)	1.02 [0.70,1.48] (n=169)	0.94 [0.73, 1.22] (n=569) p=0.65	0.95 [0.72, 1.24] (n=470) p=0.68

Table III. The proportion (percentage) of children with exacerbations stratified by treatment class and SNP rs1042713.

Treatment group	BREATHE			GALA II			PACMAN			PAGES			PASS		
	AA	AG	GG	AA	AG	GG	AA	AG	GG	AA	AG	GG	AA	AG	GG
SABA alone	7/29 (24%)	28/101 (28%)	25/88 (28%)	57/92 (62%)	150/241 (62%)	88/157 (56%)	0/10 (0%)	2/42 (5%)	0/21 (0%)	0/8 (0%)	5/23 (22%)	4/20 (20%)	0	0	0
ICS alone	52/98 (53%)	121/296 (41%)	126/304 (41%)	43/62 (69%)	93/151 (62%)	46/70 (66%)	6/76 (8%)	24/243 (10%)	19/157 (12%)	15/38 (40%)	33/122 (27%)	35/113 (31%)	1/2 (50%)	7/15 (47%)	2/11 (18%)
ICS+LABA	18/22 (82%	25/55 (46%)	33/61 (54%)	25/30 (83%)	43/56 (77%)	23/36 (64%)	6/20 (30%)	3/73 (4%)	4/54 (7%)	11/18 (61%)	36/64 (56%)	25/52 (48%)	8/17 (47%)	25/41 (61%)	16/38 (42%)
ICS+LTRA	8/11 (73%)	18/26 (69%)	15/28 (54%)	22/34 (65%)	59/81 (73%)	39/62 (63%)	1/5 (20%)	3/9 (33%)	1/0 (11%)	5/11 (46%)	16/27 (59%)	17/27 (63%)	2/5 (40%)	2/7 (29%)	10/12 (83%)
ICS+LABA+ LTRA	11/15 (73%)	23/37 (62%)	26/39 (67%)	13/16 (81%)	37/50 (74%)	25/33 (76%)	0/4 (0%)	3/21 (14%)	4/16 (25%)	14/21 (67%)	58/82 (71%)	47/66 (71%)	17/35 (49%)	58/101 (57%)	48/94 (51%)

Childhood asthma exacerbations and the Arg-16 beta2 receptor polymorphism: a meta-analysis stratified by treatment

Steve Turner MD[1], Ben Francis PhD[2], Susanne Vijverberg PhD[3], Maria Pino-Yanes PhD[4,5], Anke H Maitland-van der Zee PhD[3], Kaninika Basu MD[6], Lauren Bignell MBBS[6], Somnath Mukhopadhyay MD[6,7], Roger Tavendale PhD[7], Colin Palmer PhD[7], Daniel Hawcutt PhD[8], Munir Pirmohamed PhD[9], Esteban G Burchard MD MPH[10,11] and Brian Lipworth MD[12] on behalf of the Pharmacogenomics in Childhood Asthma consortium

[1]Child Health, University of Aberdeen

[2] Department of Biostatistics, University of Liverpool, Liverpool

[3]Division of Pharmacoepidemiology and Clinical Pharmacology, University of Utrecht

[4]CIBER de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain

[5]Research Unit, Hospital Universitario N.S. de Candelaria, Santa Cruz de Tenerife, Spain

[6]Academic Department of Paediatrics, Royal Alexandra Children's Hospital, Brighton and Sussex Medical School, Brighton, United Kingdom.

[7]Population Pharmacogenetics Group, University of Dundee

[8]Department of Women's and Children's Health, Institute of Translational Medicine, University of Liverpool, Liverpool

[9] Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine, University of Liverpool

[10] Department of Bioengineering and Therapeutic Sciences and Medicine, University of California, San Francisco, USA

[11]Center for Genes, Environment and Health, University of California, San Francisco, USA

[12] Asthma and allergy Research Group, University of Dundee

Contact details: Dr S Turner, Child Health, Royal Aberdeen Children's Hospital, Aberdeen, AB25 2ZG tel +44 1224 438475, fax +44 1224 438469, s.w.turner@abdn.ac.uk

Funding sources. There were no funds for the present analysis and the individual cohorts were funded as follows:

BREATHE was funded by Scottish Enterprise Tayside, the Gannochy Trust, the Perth and Kinross City Council and Brighton and Sussex Medical School.

GALA II. The contribution from GALA II to this work was supported by grants from National Institutes of Health to E.G.B.: the National Heart, Lung and Blood Institute (HL088133, HL078885, HL004464, HL104608 and HL117004); the National Institute of Environmental Health Sciences (ES015794); the National Institute on Minority Health and Health Disparities (MD006902); the National Institute of General Medical Sciences (GM007546). E.G.B was also funded by the American Asthma Foundation, the RWJF Amos Medical Faculty Development Award, the Sandler Foundation and the Flight Attendant Medical Research Institute. MPY was supported by a postdoctoral fellowship from Fundacion Ramon Areces (www.fundacionareces.es).

PACMAN was funded by a strategic alliance between GSK and Utrecht Institute for Pharmaceutical Sciences.

PAGES was funded by the Chief Scientist Officer for Scotland.

PASS was funded by the UK department of health through the "NHS Chair of Pharmacogenomics

None of the authors has any financial relationship with a biotechnology and/or pharmaceutical manufacturer that has an interest in the subject matter or materials discussed in the submitted manuscript.

Running head: ADRB2 and childhood asthma exacerbations

Word count for main body of manuscript: 2793

ABSTRACT

Background. The Gly-to-Arg substitution at the 16 position (rs1042713) in the beta 2 adrenoceptor (*ADRB2*) gene is associated with enhanced down-regulation and uncoupling of beta-2 receptors.

Objectives. To undertake a meta-analysis to test the hypothesis that there is an interaction between the A allele of rs1042713 (Arg16 amino acid) and long acting beta agonist (LABA) exposure for asthma exacerbations in children.

Methods. Children with diagnosed asthma were recruited in five populations (BREATHE, GALA II, PACMAN, PAGES and PASS). A history of recent exacerbation and asthma treatment were determined from questionnaire data. DNA was extracted and the Gly16Arg genotype determined.

Results. Data from 4226 children of white Northern European and Latino origin were analysed and the odds ratio for exacerbation increased by 1.52 [1.17, 1.99] p=0.0021 for each copy of the A allele among the 637 children treated with inhaled corticosteroids (ICS) plus LABA but not for treatment with ICS alone (n=1758), nor ICS plus leukotriene receptor antagonist (LTRA, n=354) or ICS plus LABA plus LTRA (n=569).

Conclusions. The use of LABA as "add-on controller", but not LTRA, is associated with increased risk of asthma exacerbations in children carrying one or two A alleles at rs1042713. Prospective genotype stratified clinical trials are now required to explore the potential role of rs1042713 genotyping for personalised asthma therapy in children.

Key words: Adrenergic receptors; Asthma; Child; Disease exacerbation; Therapeutics.

79 KEY MESSAGE

80 Clinical trials are required to determine whether treatment stratified by rs1042713 will
81 reduce asthma exacerbation risk in children with one or two A alleles.

82

CAPSULE SUMMARY

This meta-analysis finds increased asthma exacerbation risk for children who carry ≥ 1 A allele of rs1042713, but only in the context of treatment with inhaled corticosteroids and long acting beta agonist.

88 **ABBREVIATIONS**

89 ADRB2 Beta 2 Adrenoceptor

90 LABA Long Acting Beta Agonists

91 LTRA Leukotriene Receptor Antagonist

92 ICS Inhaled Corticosteroids

93 MAF Minor Allele Frequency

94 PAGES Paediatric Asthma Gene Environment Study

95 PASS Pharmaco genetics of Adrenal Suppression with inhaled Steroid study

96 SABA Short Acting Beta Agonists

97 SNP Single Nucleotide Polymorphism

98

INTRODUCTION

Asthma is a common condition in children where there is heterogeneity in response to treatment with inhaled corticosteroids (ICS), long acting beta agonists (LABA) and leukotriene receptor antagonists (LTRA) [1, 2]. Some of this heterogeneity may reflect genetic variations within the population, and variants in the gene coding for the beta 2 adrenoceptor (*ADRB2*) have been associated with increased risk for symptoms [3-5]. Of particular interest is the single nucleotide polymorphism (SNP) rs1042713, a Gly to Arg amino acid substitution at the position 16 of the *ADRB2* gene, which has been associated with differences in pulmonary function responsiveness to short acting beta agonists in children [6-9] and the underlying mechanism of enhanced down-regulation and uncoupling of beta-2 receptors is thought to reflect an altered response to short and long acting beta agonists (SABA and LABA).

Although the SNP rs1042713 appears to alter physiological and clinical response to SABA and LABA in paediatric populations, the clinical relevance of this association remains unclear. In two clinical trials there was no evidence for an association between the A allele of rs1042713 (Arg16 amino acid) and increased symptom score [1, 7]. There is inconsistent evidence from observational studies that this SNP may be relevant to exacerbations. In children, the homozygous G/G genotype of rs1042713 has been linked with increased risk for hospitalisation[10], reduced bronchodilator response to short acting beta agonists[9], prolonged stay in hospital [11] and intensive care unit stay [12] after presentation with acute asthma, whilst the heterozygous genotype of rs1042713 has been linked with increased risk for intubation for acute asthma [13]. Two other groups have observed associations between A/A genotype of rs1042713 and increased exacerbations among those treated with LABA [3, 4]

but this was not confirmed in a third population[14]. These studies have also observed increased exacerbation risk [3] and poorer asthma control [4] among those children homozygous for A/A for the SNP rs1042713 in receipt of ICS (but not LABA). In one study [3], there was evidence that concomitant LTRA treatment might negate any increased risk for exacerbation associated with LABA treatment, while those children who are homozygous for Arg16 had better asthma outcomes when treated with LTRA rather than LABA in addition to to ICS [15]. Prospective studies undertaken in adult populations have found no evidence for LABA treatment being associated with adverse outcomes when added to ICS[16-18]

To better understand the interactions between the SNP rs1042713 of *ADRB2* and asthma treatment, we undertook a meta-analysis of results from five previously described populations [19]. Our hypothesis was that there is an interaction between the A allele of rs1042713 (Arg16 amino acid) and treatment with LABA, but not LTRA, for asthma exacerbation risk and that this risk might be further increased by exposure to daily SABA.

METHODS

Study design. Children with asthma were recruited to five cross-sectional studies (BREATHE, GALA II, PAGES, PACMAN and PASS). The BREATHE and PAGES populations were recruited from primary and secondary care in Scotland, PACMAN was recruited from children attending community pharmacists in Netherlands, GALA II recruited children in USA and Puerto Rico who had four Latino grandparents and PASS recruited children with asthma who had adrenal suppression testing in 25 hospitals across the UK. Further details of the study population's recruitment are presented in the Online Repository. DNA was extracted from saliva or blood and the genotypes for rs1042713 determined. The primary outcome was asthma exacerbation (with reference to six months in BREATHE, PAGES and PASS and 12 months in GALA II and PACMAN). Asthma treatment was categorised thus: (i) as required (prn) SABA but no preventer treatment (ii) ICS monotherapy plus prn SABA (iii) ICS plus prn SABA and LABA (iv) ICS plus prn SABA and LTRA and (v) ICS plus prn SABA, LABA and LTRA. As previously [5], as required SABA was categorised as at least once daily or less frequently. Approval was obtained from medical research ethics committees from each institute prior to recruitment. All participants gave verbal assent and parents or participants gave written consent as appropriate.

Definitions of exacerbation. For BREATHE and PAGES the definition of exacerbation was at least one of the following in the previous six months in the context of asthma symptoms: hospital admission, course of oral steroids or absence from school. For GALA, an exacerbation was defined as at least one of the following during the previous 12 months: oral corticosteroid rescue treatment, hospitalisation or need to seek emergency asthma care. For PACMAN, an exacerbation was defined as an asthma-related visit to emergency

department and/or prescribed a course of oral steroids in the past twelve months. The definition of exacerbation for PASS was at least one course of rescue oral steroids in the previous six months.

DNA collection, extraction and analysis. For BREATHE, PACMAN and PAGES, saliva was collected in commercially available pots (Oragene, DNA Genotech, Ontario, Canada) DNA was prepared using the Qiagen Dneasy 96 kit, and genotypes were determined in the Dundee laboratory using Taqman based allelic discrimination assays on an ABI 7700 sequence detection system, as described previously[3]. For GALA II, DNA was extracted from whole blood and the Axiom® LAT1 array (World Array 4, Affymetrix, Santa Clara, CA) was used to determine genome wide-genotype data as described elsewhere[20]. For PASS, the Illumina Human OmniExpressExome-8 v1.0 chip (Illumina, San Diego, CA) was used for genotyping.

Statistical analysis. The primary outcome was recent exacerbation and this was related to genotype in logistic models. An additive model [3] was used, i.e. a gene/dosage effect for the A allele [Arg16 aminoacid] which adjusted for confounders (i.e. gender, age, second hand smoke exposure [3]). Each population was stratified by treatment and risk for exacerbation per genotype was calculated in each treatment group. Daily SABA use was recorded for BREATHE, PACMAN and PAGES and here an interaction as sought for SABA treatment x genotype. Regression analyses in GALA II included the same covariates as in the other studies, but additionally we included estimates of global African and Native American genetic ancestry to avoid confusion due to population stratification. Standard statistical software was used (SPSS version 22.0.0.1). The meta-analysis of data from the five populations was performed using a fixed-effect (inverse variance–weighted) model, where

183 the effect size estimates, *β* coefficients, are weighted by their estimated standard errors
184 using GWAMA software[21]. We estimated the power of the study to detect the associations
185 with exacerbations following the methodology of Purcell *et al* [22]. Our power calculations
186 provide the maximal power we could obtain from the meta-analysis of the cohorts at the
187 significance level of 5%. The odd ratios of 1.2, 1.5 and 3 were selected based on initial
188 results from the BREATHE trial. With the exception of the ICS+LTRA treatment group, all
189 strata were sufficiently powered to detect an odds ratio of 1.5 or above (See table I in the
190 Online Repository) . Forest plots were generated with the package *rmeta* for R. A p value of
191 <0.05 was assumed to be significant.

192

RESULTS

Study subjects

Genotype, treatment and exacerbation data were available in 4226 children including 1210 from BREATHE, 1171 from GALA II, 760 from PACMAN, 695 from PAGES and 390 from PASS, table I. The Gly16Arg polymorphism was in HWE for all cohorts with the exception of BREATHE (exact test p=0.012) considered as a whole, but it was in HWE in the group of children that did not have exacerbations (p=0.624). The minor allele frequency (MAF) for GALA II was higher when compared to the three UK cohorts (0.45 vs 0.37) $p=1x10^{-10}$, and intermediate for the PACMAN population. Regardless of treatment, across the five populations the additive model found an increased risk for exacerbation for each copy of the A allele amounting to 1.11 [1.01, 1.22] p=0.035, n=4226 (See table II in the Online Repository).

Risk of exacerbation across maintenance treatment groups

The odds ratio for exacerbation was 1.52 [1.17, 1.99] for each copy of the A allele among the 637 children treated with ICS plus LABA, table II. The risk for exacerbation was not increased among other treatment groups, table II. Table III presents the proportion of children with exacerbations stratified by population, treatment and genotype. The analysis for children treated with ICS plus LABA had >90% power to detect an association with increased risk for exacerbation at the significance level of 5% (Table I in the Online Repository). An analysis of local African ancestry at the Gly16Arg locus was undertaken in the GALA II population to examine if the number of chromosomes indicative of African ancestry at this locus was associated with increased exacerbations. There was no

association of local African ancestry with exacerbations in GALA II in the overall population (OR=1.17, 95% CI: 0.89-1.53, *p*=0.270) or in the group of patients treated with ICS plus LABA (OR=1.78, 95% CI: 0.58-5.49, *p*=0.316).

Risk of exacerbation in relation to SABA use

Among the 822 children in receipt of daily SABA (including 56 who were not on ICS, LABA or LTRA) there was no evidence of increased risk in the additive model (OR 1.01 [0.79, 1.31], see table III in the Online Repository)). Among those children in receipt of ICS plus LABA there was no evidence of any additional increased risk in relation to each A allele for exacerbations among those taking daily SABA (see table IV in the Online Repository).

Asthma control scores and Arg16 homozygous genotype

The risk for poorly controlled asthma (as evidence by the asthma control questionnaire 6 score >1.5) was increased among A/A homozygotes prescribed ICS only within the cohort PACMAN (OR 2.15) [(4)]. Within the PAGES population 63% (282/446) were poorly controlled (as evidenced by Children's Asthma Control Test score <20) and there was no increase in risk for poor control for A/A homozygotes among any treatment groups.

DISCUSSION

Genetic epidemiology is complicated by inconsistent findings between populations. Therefore replication of findings across different populations is crucial to generalising results [23]. Associations between SNP rs1042713 and LABA and SABA treatment have been previously reported in evaluations of the first 546 children recruited to BREATHE[3] and the first 597 recruited to PACMAN[4] (data from 1210 and 760 included in the present report respectively). However, the results of other studies in adults were in apparent conflict with the above observations. This meant that, prior to this study, the important clinical question of whether or not there is a need to progress to further randomised controlled trials assessing benefit with testing for SNP rs1042713 in the clinical setting had not been resolved. This study combined data from five cohorts of children with asthma from white European and Hispanic/Latino populations to explore interactions between exposures to different asthma medications and the SNP rs1042713 for risk of asthma exacerbation. We analysed data from 4226 children and draw three conclusions. First, among children exposed to ICS plus LABA as dual combination therapy there was a 52% increased risk for exacerbation for each copy of the A allele. Second, the interaction between the A allele and exposure to LABA was not present when LTRA treatment was also co-prescribed as triple therapy. Third, there was no evidence that daily SABA usage in addition to ICS plus LABA was associated with any increased further risk for exacerbation among children carrying at least one A allele. The combined incidence of A/G heterozygous and A/A homozygous genotype is approximately 60% and these observations implicate the SNP rs1042713 as an important factor in the well-recognised heterogeneity of treatment response in children with asthma[1, 2]. This study has established the need for further prospective clinical trials where treatment is stratified by genotype to

move these observations into clinical practice in order to evaluate a more personalised approach to treatment of children with poorly controlled asthma despite treatment with inhaled corticosteroids.

We observed heterogeneity between populations for the relationship between SNP rs1042713 and treatment with ICS plus LABA and risk for exacerbation with the risk being highest in GALA II and lowest in PASS. Although this study was not designed to explain the variability between populations, there was no obvious association between the effect size for exacerbation risk associated with A allele and characteristics of the five populations; for example the children in GALA II and PASS were comparable in terms of age, sex distribution and exacerbation rate. More children in PASS were in receipt of ICS plus LABA compared to GALA II but the hypothesis that exacerbation risk attributable to the A allele is lower for populations where LABA treatment is more prevalent is not supported by observations in the PACMAN and PAGES populations where 19% in each received LABA but the exacerbation risk associated with ICS plus LABA was 2.54 and 1.29 respectively. The heterogeneity between populations, and within populations [1, 2], might give potential insight into the pharmacogenetic mechanism(s) but also highlights the need for stratified treatment in childhood asthma.

The minor allele frequency was substantially higher for children in the GALA II population compared to the three UK populations and, as suggested by previous work [18, 24], we explored the possibility that the increased exacerbation rate associated with the Arg16 allele in LABA-treated GALA II subjects reflected the African ancestry associated with this allele. In our adjustment for measures of ancestry for the analyses of the Gly16Arg locus within the GALA II population we did not find significant evidence that African ancestry was

277 relevant to the positive correlation between minor allele frequency and prevalence of
278 exacerbation, however our analysis was underpowered and the two-fold increase in risk
279 which was detected might have been significant had our sample size been larger. Our study
280 was not designed to explore how ethnic differences might be relevant to the
281 pharmacogenetics or treatment response to LABA and unfortunately there were insufficient
282 numbers of children with African ancestry in the cohorts other than GALA II to further
283 explore this intriguing hypothesis which merits focussed research in future.

284 The pharmacogenetics of LABA and SABA are notable for the contrasting effects seen for the
285 Gly16Arg locus on acute versus chronic SABA. There has been considerable consistency in
286 the observed effects of Gly16Arg on acute SABA response with many studies showing a
287 similar direction of effect on bronchodilation (favouring Arg16)[6, 7, 11, 25, 26]. A seemingly
288 opposite effect (favouring Gly16) was seen for chronic SABA exposure and lung function and
289 asthma control in the Beta Agonists in Mild Asthma (BAGS) [27] and Beta-Adrenergic
290 Response by Genotype (BARGE) [28] studies and another study by Taylor et al [29]. The focus
291 of the present study was LABA therapy but we found no evidence for either daily SABA use
292 or the combination of daily SABA plus LABA being linked with increased exacerbation risk for
293 children carrying Arg16 allele. One interpretation of our findings is that the LABA caused
294 effective adrenoceptor blockade and the “adverse” effect of LABA treatment had subsumed
295 any “benefit” of acute SABA treatment for individuals carrying an Arg16 allele.

296

297 Although we find evidence here for response to LABA therapy to be modified by the
298 Gly16Arg locus in children, for adults this locus appears to have no effect on response to
299 LABA therapy based on large retrospective analyses and prospective genotype-stratified

clinical trials by Bleecker [16] and the LARGE trial [18]. There is evidence that the Gly16 allele might be associated with a bronchoprotective effect in association with LABA treatment [18, 30]. The present study was not designed to explore the apparent inconsistency between observations in adults and children but differences in response of children and adults to LABA are well-recognised. The addition of LABA to ICS treatment in adults with poorly controlled asthma is accepted to be superior to alternative treatments [31, 32] but in children the evidence is that LABA are no more effective that addition of LTRA or increase in ICS [1, 33].

Our analysis included a relatively large number of well-characterised children with asthma from five populations and therefore any significant association is likely to be generalizable to other similar populations, perhaps aside from those where there is a higher proportion of individuals of African descent where the A allele is more prevalent. In the literature there are apparently conflicting associations reported between variants of the SNP rs1042713 and response to treatment [3-6, 9, 11] and one explanation for this heterogeneity is that the earlier findings are based on relatively small single populations, i.e. which number less than 1000, and meta-analysis addresses the potential for false positive findings and/or associations which are idiosyncratic for one population. This meta-analysis confirms previously reported increases in the risk for exacerbations among Arg16 homozygotes in receipt of LABA treatment [3, 4]. The present study failed to replicate previously reported associations between homozygous genotype A/A and treatment with ICS alone and increased risk for exacerbation [3] or poor asthma control [4], suggesting the possibility of false positive findings. The magnitude of risk for exacerbation associated with ICS and LABA treatment and A allele reported in first 546 children recruited to the BREATHE cohort [3] is slightly

reduced in the larger population (2.1 versus 1.5) but remains significant across all five populations. The five populations included were heterogeneous for asthma outcomes and the results could be generalised to Western European and Hispanic/Latino populations but, given the potential for different associations between rs1042713 and asthma treatment response between different ethnic groups [26], our results may not be relevant to all populations.

In our analysis we explored the possible additive effect of treatment with daily SABA and LABA for exacerbations, and we have previously reported that either treatment is associated with increased exacerbations for the BREATHE population among Arg/Arg homozygotes[5]. When data were pooled there was no apparent additive effect of SABA on LABA for exacerbation risk for children with one or two Arg alleles. These results should be treated with caution since, even with a relatively large population such as we present here, there were relatively few children with ICS plus LABA and daily SABA exposure and the analysis was probably underpowered and there may be a modest additive effect which we were not able to detect.

The mechanism(s) underlying the association between the Arg16 allele and increased exacerbation in the context of LABA treatment are thought to be mediated by enhanced agonist induced down-regulation and receptor uncoupling, resulting in subsensitivity of response [34, 35]. Our novel finding of no increased risk for exacerbation among A/A homozygotes in receipt of ICS and LABA and LTRA suggests that factors other than *ADRB2* down regulation are active since LABA exposure in this group of children might be expected to down regulate *ADRB2*. It is likely that LTRA merely confer an additional anti-inflammatory effect in individuals exposed to LABA, such that in genetically susceptible

346 patients it might be seen as a salutary effect by counteracting the response to LABA. Where
347 the LABA effect is negated (i.e. in Arg/Arg) the additive effect of LTRA will be more evident
348 compared to the setting where LABA effect is more pronounced (i.e. Gly/Gly) and the
349 additive effect of LTRA will be less evident. Indeed in one study using AMP challenge as the
350 primary outcome, there was better protection with ICS/LABA/LTRA as triple therapy
351 compared to dual therapy with ICS/LABA, which was also mirrored by effects on exhaled NO
352 and blood eosinophils, suggesting that the apparent counteracting role of LTRA may arise
353 from the additional anti-inflammatory effects of LTRA [(36)].

354 This study has a number of limitations, which should be considered when these results are
355 interpreted. First, the associations described here do not imply causation but the findings
356 are consistent with the results of a small genotype stratified randomised controlled trial,
357 which found favourable outcomes among A/A homozygotes taking LTRA compared to LABA
358 over a period of 12 months when used as add on therapy to ICS [(15)]. Second, although we
359 are able to be conclusive as to the nature of the relationship between exposure to LABA and
360 A/A homozygous genotype and exacerbation, we cannot exclude the possibility that there
361 may be a small additive relationship between SABA and LABA for exacerbation. The
362 relationship between LABA, SABA and exacerbations will always be a challenge to study
363 since frequent SABA treatment is an indication for LABA therapy but our findings suggest
364 that the magnitude of association with LABA is greater than SABA, which perhaps is not
365 surprising given the potential impact more prolonged receptor occupancy conferred by
366 LABA than SABA, especially in genetically susceptible individuals. Third, more detailed
367 genotyping of the ADRB2 locus or haplotype analysis might have yielded additional insight
368 into the relationship between genetic variations of the *ADRB2* but neither GWAS or

haplotype data were available for all cohorts and we focussed on the SNP rs1042713 since there is a large literature which indicates that this is associated with outcomes for asthma treatment. A fourth limitation is that we have assumed that treatment has been assigned based on the same criteria and it is possible that in some cohorts, children with more severe asthma and at increased risk for exacerbations might not have received LABA treatment but this would tend to underestimate the effect of the interaction between LABA treatment and the SNP rs1042713 for exacerbations. A fifth limitation is that rare variants were not genotyped which could also have an impact on adverse events during LABA therapy; however, these cohorts were not powered for a rare variant analysis and the rare variants identified by Ortega *et al* [(38)] occurred in the background of Gly16, not Arg16. Finally, the polymorphism Gly16Arg allele frequency was not in Hardy Weinberg Equilibrium for the whole BREATHE cohort but the consistency of the results across the cohorts suggests that the deviation within this single cohort did not substantially influence the overall results. Furthermore, Gly16Arg is an inconsistently replicated and, at best, weak locus for asthma severity so it does not seem plausible that selection for exacerbation (the primary outcome and linked to asthma severity) in this study is the cause for the deviation from HWE seen in the BREATHE cohort. Since Gly16Arg is a better established pharmacogenetic locus, a more plausible outcome which would influence deviations from HWE is exacerbations or symptoms despite frequent SABA use or LABA use, but this represents a small percentage of the BREATHE cohort (18% and 11%, respectively).

In conclusion, children with asthma on ICS plus LABA were at 52% increased risk for exacerbation in the previous 6-12 months for each A allele (Arg16 amino acid) compared to G/G homozygotes (Gly16/Gly16). Knowing that there are 1 million children in the UK with asthma and 10% of these are prescribed LABA [(39)] and 60% of these carry at least one Arg16 allele, there are approximately 60,000 children in the UK today, and approximately 25,000 in

393 the Netherlands, who might be at risk from the morbidity of exacerbation which is
394 preventable by treatment with either no LABA or perhaps with the addition of LTRA if
395 genotyping of rs1042713 could be made available at the point of prescribing. Put another
396 way, and assuming that at least one third of the UK national healthcare costs attributable to
397 childhood asthma are due to urgent care[40, 41], stratified treatment might reduce the annual
398 direct costs to the UK National Health Service for the management of childhood asthma
399 exacerbations by 10% (i.e. a 50% reduction in 60% of the population). Whilst there are no
400 recent published costs for the management of childhood asthma, costs in the US have been
401 estimated at $791per child per annum in 2005 [41] and to total £150 million in the UK in
402 1997 [42]. It is thus likely that stratified treatment in childhood asthma will save tens of
403 millions of pounds in direct healthcare costs. Not all asthmatics who inherit an A allele will
404 experience increased morbidity with LABA treatment and this might be explained by rare
405 variants, perhaps at the same locus [38], pathway related variation[43], epigenetic
406 mechanisms or treatment adherence.

REFERENCES

1. Lemanske RF Jr. Mauger DT. Sorkness CA. Jackson DJ. Boehmer SJ. Martinez FD. Strunk RC. Szefler SJ. Zeiger RS. Bacharier LB. Covar RA. Guilbert TW. Larsen G. Morgan WJ. Moss MH. Spahn JD. Taussig LM. Childhood Asthma Research and Education (CARE) Network of the National Heart, Lung,and Blood Institute. Step-up therapy for children with uncontrolled asthma receiving inhaled corticosteroids. N Engl J Med 2010;362:975-85.

2. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC*, et al*. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-42.

3. Palmer CN, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax. 2006;61:940-4.

4. Zuurhout MJ, Vijverberg SJ, Raaijmakers JA, Koenderman L, Postma DS, Koppelman GH*, et al*. Arg16 ADRB2 genotype increases the risk of asthma exacerbation in children with a reported use of long-acting beta2-agonists: Results of the PACMAN cohort. Pharmacogenomics 2013;14:1965-71.

5. Basu K, Palmer CN, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic beta(2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. J Allergy Clin Immunol 2009;124:1188,94.e3.

6. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J Clin Invest 1997;100:3184-8.

7. Silverman EK, Kwiatkowski DJ, Sylvia JS, Lazarus R, Drazen JM, Lange C*, et al*. Family-based association analysis of beta2-adrenergic receptor polymorphisms in the childhood asthma management program. J Allergy Clin Immunol 2003;112:870-6.

8. Turner SW. Genetic predictors of response to therapy in childhood asthma. Mol Diag Ther 2009;13:127-35.

9. Finkelstein Y, Bournissen FG, Hutson JR, Shannon M. Polymorphism of the ADRB2 gene and response to inhaled beta- agonists in children with asthma: A meta-analysis. J Asthma 2009;46:900-5.

10. Turner SW, Khoo SK, Laing IA, Palmer LJ, Gibson NA, Rye P*, et al*. beta2 adrenoceptor Arg16Gly polymorphism, airway responsiveness, lung function and asthma in infants and children. Clin Exp Allergy 2004;34:1043-8.

11. Carroll CL, Stoltz P, Schramm CM, Zucker AR. Beta2-adrenergic receptor polymorphisms affect response to treatment in children with severe asthma exacerbations. Chest 2009;135:1186-92.

12. Carroll CL, Sala KA, Zucker AR, Schramm CM. Beta-adrenergic receptor polymorphisms associated with length of ICU stay in pediatric status asthmaticus. Pediatr Pulmonol 2012;47:233-9.

13. Carroll CL, Sala KA, Zucker AR, Schramm CM. beta2-adrenergic receptor haplotype linked to intubation and mechanical ventilation in children with asthma. J Asthma 2012;49:563-8.

14. Giubergia V, Gravina L, Castanos C, Chertkoff L. Influence of beta(2)-adrenergic receptor polymorphisms on asthma exacerbation in children with severe asthma regularly receiving salmeterol. Ann Allergy Asthma Immunol 2013;110:156-60.

15. Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA*, et al*. Tailored second-line therapy in asthmatic children with the arg(16) genotype. Clin Sci 2013;124:521-8.

16. Bleecker ER, Postma DS, Lawrance RM, Meyers DA, Ambrose HJ, Goldman M. Effect of ADRB2 polymorphisms on response to long acting beta2-agonist therapy: A pharmacogenetic analysis of two randomised studies. Lancet 2007;370:2118-25.

17. Bleecker ER, Nelson HS, Kraft M, Corren J, Meyers DA, Yancey SW*, et al*. Beta2-receptor polymorphisms in patients receiving salmeterol with or without fluticasone propionate. Am J Respir Crit Care Med 2010;181:676-87.

18. Wechsler ME. Kunselman SJ. Chinchilli VM. Bleecker E. Boushey HA. Calhoun WJ. Ameredes BT. Castro M. Craig TJ. Denlinger L. Fahy JV. Jarjour N. Kazani S. Kim S. Kraft M. Lazarus SC. Lemanske RF Jr. Markezich A. Martin RJ. Permaul P. Peters SP. Ramsdell J. Sorkness CA. Sutherland ER. Szefler SJ. Walter MJ. Wasserman SI. Israel E. National Heart,Lung and Blood Institute's Asthma Clinical Research Network. Effect of beta2-adrenergic receptor polymorphism on response to long acting beta2 agonist in asthma

(LARGE trial): A genotype-stratified, randomised, placebo-controlled, crossover trial. Lancet 2009;374:1754-64.

19. Vijverberg SJ, Koster ES, Tavendale R, Leusink M, Koenderman L, Raaijmakers JA*, et al*. ST13 polymorphisms and their effect on exacerbations in steroid-treated asthmatic children and young adults. Clin Exp All 2015;45:1051-9.

20. Galanter JM, Gignoux CR, Torgerson DG, Roth LA, Eng C, Oh SS*, et al*. Genome-wide association study and admixture mapping identify different asthma-associated loci in Latinos: The genes-environments and admixture in Latino Americans study. J Allergy Clin Immunol. 2014;134:295-305.

21. Magi R, Morris AP. GWAMA: Software for genome-wide association meta-analysis. BMC Bioinformatics 2010;11:288.

22. Purcell S, Cherny SS, Sham PC. Genetic power calculator: Design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149-50.

23. Hall IP, Blakey JD. Genetic association studies in thorax. Thorax 2005;60:357-9.

24. Ortega VE, Meyers DA. Pharmacogenetics: Implications of race and ethnicity on defining genetic profiles for personalized medicine. J Allergy Clin Immunol 2014;133:16-26.

25. Lima JJ, Thomason DB, Mohamed MH, Eberle LV, Self TH, Johnson JA. Impact of genetic polymorphisms of the beta2-adrenergic receptor on albuterol bronchodilator pharmacodynamics. Clin Pharmacol Therap 1999;65:519-25.

26. Choudhry S, Ung N, Avila PC, Ziv E, Nazario S, Casal J*, et al*. Pharmacogenetic differences in response to albuterol between Puerto Ricans and Mexicans with asthma. Am J Respir Crit Care Med 2005;171:563-70.

27 Israel E, Drazen JM, Liggett SB, Boushey HA, Cherniack RM, Chinchilli VM*, et al*. The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma. Am J Respir Crit Care Med 2000;162:75-80

27. Israel E. Chinchilli VM. Ford JG. Boushey HA. Cherniack R. Craig TJ. Deykin A. Fagan JK. Fahy JV. Fish J. Kraft M. Kunselman SJ. Lazarus SC. Lemanske RF Jr. Liggett SB. Martin RJ. Mitra N. Peters SP. Silverman E. Sorkness CA. Szefler SJ. Wechsler ME. Weiss ST. Drazen JM. National Heart, Lung,and Blood Institute's Asthma Clinical Research Network. Use of regularly scheduled albuterol treatment in asthma: Genotype-stratified, randomised, placebo-controlled cross-over trial. Lancet 2004;364:1505-12.

28. Taylor DR, Drazen JM, Herbison GP, Yandava CN, Hancox RJ, Town GI. Asthma exacerbations during long term beta agonist use: Influence of beta(2) adrenoceptor polymorphism. Thorax 2000;55:762-7.

29. Lee DK, Currie GP, Hall IP, Lima JJ, Lipworth BJ. The arginine-16 beta2-adrenoceptor polymorphism predisposes to bronchoprotective subsensitivity in patients treated with formoterol and salmeterol. Br J Clin Pharmacol 2004;57:68-75.

30. British Thoracic Society/Scottish Intercollegiate Guidelines Network. British guideline on the management of asthma 2014. (accessed 2 June 2015 at https://www.brit-

thoracic.org.uk/document-library/clinical-information/asthma/btssign-asthma-guideline-2014/)

31. Global Initiative for Asthma. Pocket guide for asthma management and prevention. (accessed 2 June 2015 at http://www.ginasthma.org/documents/1)

32. Vaessen-Verberne AA, van den Berg NJ, van Nierop JC, Brackel HJ, Gerrits GP, Hop WC*, et al*. Combination therapy salmeterol/fluticasone versus doubling dose of fluticasone in children with asthma. Am J Respir Crit Care Med 2010;182:1221-7.

33. Ellsworth DL, Coady SA, Chen W, Srinivasan SR, Elkasabany A, Gustat J*, et al*. Influence of the beta2-adrenergic receptor Arg16Gly polymorphism on longitudinal changes in obesity from childhood through young adulthood in a biracial cohort: The Bogalusa heart study. Int J Obesity Rel Metabol Dis 2002;26:928-37.

34. Lipworth B. Beta-adrenoceptor genotype and bronchoprotective subsensitivity with long-acting beta-agonists in asthma. Am J Respir Crit Care Med 2013;188:1386-7.

35. Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of beta 2-adrenergic receptor genotypes on signal transduction in human airway smooth muscle cells. Am J Respir Cell Mol Biol 1995;13:25-33.

36. Currie GP, Lee DK, Haggart K, Bates CE, Lipworth BJ. Effects of montelukast on surrogate inflammatory markers in corticosteroid-treated patients with asthma. Am J Respir Crit Care Med 2003;167:1232-8.

37. Ambrose HJ, Lawrance RM, Cresswell CJ, Goldman M, Meyers DA, Bleecker ER. Effect of beta2-adrenergic receptor gene (ADRB2) 3' untranslated region polymorphisms on inhaled corticosteroid/long-acting beta2-adrenergic agonist response. Respir Res 2012;13:37.

38. Ortega VE, Hawkins GA, Moore WC, Hastie AT, Ampleford EJ, Busse WW*, et al*. Effect of rare variants in ADRB2 on risk of severe exacerbations and symptom control during longacting beta agonist treatment in a multiethnic asthma population: A genetic study. Lancet Respir Med 2014;2:204-13.

39. Turner S, Thomas M, von Ziegenweidt J, Price D. Prescribing trends in asthma: A longitudinal observational study. Arch Dis Child 2009;94:16-22.

40. Lozano P, Fishman P, VonKorff M, Hecht J. Health care utilization and cost among children with asthma who were enrolled in a health maintenance organization. Pediatr1997;99:757-64.

41. Wang LY, Zhong Y, Wheeler L. Direct and indirect costs of asthma in school-age children. Prevent Chronic Dis 2005;2:A11.

42. Lenney W. The burden of pediatric asthma. Pediatr Pulmonol 1997;15:13-6..

43. Kim SH, Ye YM, Lee HY, Sin HJ, Park HS. Combined pharmacogenetic effect of ADCY9 and ADRB2 gene polymorphisms on the bronchodilator response to inhaled combination therapy. J Clin Pharm Therap 2011;36:399-405.

Table I. Comparison of details of children in each of the five study populations. *Dutch, Moroccan and Turkish ethnicities were considered Caucasian in PACMAN. †ethnicity data were not available for all participants in PAGES. ‡daily short acting beta agonist status was not determined in GALA II and PASS.¶ minor allele frequency = frequency of minor allele homozygous genotype + (frequency of heterozygous genotype)/2

		BREATHE n=1210	GALA II n=1171	PACMAN n=760	PAGES n=695	PASS n=390
	% Male (n)	60% (725)	58% (676)	63% (478)	57% (399)	56% (172)
	% Exposed to tobacco smoke at home (n)	35% (424)	21% (242)	14% (108)	21% (146)	36% (108)
	Mean age, (SD) years	9.7 (3.8)	11.9 (2.7)	8.7 (2.3)	9.8 (3.7)	11.1 (4.0)
	Recent exacerbation	44% (536)	65% (763)	10% (76)	47% (323)	75% (295)
Ethicity	% Caucasian	No data	0%	90%* (681/753)	93%† (358/384)	99% (388/390)
	% Hispanic		100%	0.4% (3/753)	0%	0%
	% African		0%	1% (8/753)	0%	0%
	% Other (including mixed)		0%	8.6% (61/753)	7% (26/384)	1% (2/390)
	Minor allele frequency¶	0.37	0.45	0.41	0.37	0.38
	A/A (Arg/Arg) (n)	15% (175)	20% (234)	15% (115)	14% (96)	16% (61)

rs1042713 genotype	A/G (Arg/Gly) (n)	43% (515)	49% (579)	51% (388)	46% (321)	43% (169)
	G/G (Gly/Gly) (n)	43% (520)	31% (358)	34% (257)	40% (278)	41% (160)
Treatment group	% Short acting beta agonist alone (n)	18% (218)	42% (490)	10% (73)	7% (51)	0%
	% Inhaled corticosteroid, ICS, (n)	58% (698)	24% (283)	63% (476)	40% (273)	7% (28)
	% ICS plus long acting beta agonist, LABA, (n)	11% (138)	10% (122)	19% (147)	19% (134)	33% (96)
	%ICS plus leukotriene receptor antagonist, LTRA, (n	5% (65)	15% (177)	3% (23)	9% (65)	8% (24)
	% ICS plus LABA plus LTRA, (n)	8% (91)	9% (99)	5% (41)	24% (169)	59% (230)
% with daily short acting beta agonist dosing, (n)		21% (250)	‡	49% (364)	30% (209)	‡

Table II. Odds ratio for exacerbation per copy of the A allele (Arg16 amino acid). Results are from logistic regression models which adjusted for sex, age and exposure to second hand smoke at home. SABA=short acting beta agonist. ICS =inhaled corticosteroids. LABA= long acting beta agonist. LTRA=leukotriene receptor antagonist. *There were no children in PASS on SABA alone.

Treatment group	Odds ratio [95% confidence interval] for exacerbation per A allele (referenced to none)						
	BREATHE	GALA II	PACMAN	PAGES	PASS	Results from all cohorts combined	Results for all cohorts except GALA II
SABA alone	0.87 [0.54, 1.40] (n=218)	1.08 [0.81,1.43] (n=490)	1.07 [0.06, 20.2] (n=73)	0.71 [0.18, 2.80] (n=51)	*	1.01 [0.79, 1.28] (n=832) p=0.95	0.85 [0.55, 1.33] (n=342) p=0.49
ICS alone	1.15 [0.92, 1.43] (n=698)	1.12 [0.78,1.62] (n=283)	0.83 [0.53, 1.31] (n=476)	1.17 [0.80, 1.71] (n=273)	4.81 [0.79, 29.33] (n=28)	1.11 [0.95, 1.31] (n=1758) p=0.18	1.11 [0.94, 1.33] (n=1475) p=0.22
ICS+LABA	1.52 [0.92, 2.50] (n=138)	2.07 [1.03, 4.16] (n=122)	2.54 [1.06, 6.06] (n=147)	1.29 [0.76, 2.19] (n=134)	1.21 [0.68, 2.14] (n=96)	1.52 [1.17, 1.99] (n=637) p=0.0021	1.44 [1.08, 1.93] (n=515) p=0.01
ICS+LTRA	1.86 [0.85, 4.08] (n=65)	1.26 [0.79, 2.02] (n=177)	2.10 [0.43, 10.2] (n=23)	0.69 [0.34, 1.39] (n=65)	0.31 [0.08, 1.18] (n=24)	1.11 [0.80, 1.55] (n=354) p=0.52	0.98 [0.61, 1.56] (n=177) p=0.93
ICS+LABA+LTRA	1.03 [0.54,1.96] (n=91)	0.93 [0.36,2.39] (n=99)	0.23 [0.04,1.46] (n=41)	0.87 [0.52, 1.45] (n=169)	1.02 [0.70,1.48] (n=169)	0.94 [0.73, 1.22] (n=569) p=0.65	0.95 [0.72, 1.24] (n=470) p=0.68

Table III. The proportion (percentage) of children with exacerbations stratified by treatment class and SNP rs1042713.

Treatment group	BREATHE			GALA II			PACMAN			PAGES			PASS		
	AA	AG	GG	AA	AG	GG	AA	AG	GG	AA	AG	GG	AA	AG	GG
SABA alone	7/29 (24%)	28/101 (28%)	25/88 (28%)	57/92 (62%)	150/241 (62%)	88/157 (56%)	0/10 (0%)	2/42 (5%)	0/21 (0%)	0/8 (0%)	5/23 (22%)	4/20 (20%)	0	0	0
ICS alone	52/98 (53%)	121/296 (41%)	126/304 (41%)	43/62 (69%)	93/151 (62%)	46/70 (66%)	6/76 (8%)	24/243 (10%)	19/157 (12%)	15/38 (40%)	33/122 (27%)	35/113 (31%)	1/2 (50%)	7/15 (47%)	2/11 (18%)
ICS+LABA	18/22 (82%	25/55 (46%)	33/61 (54%)	25/30 (83%)	43/56 (77%)	23/36 (64%)	6/20 (30%)	3/73 (4%)	4/54 (7%)	11/18 (61%)	36/64 (56%)	25/52 (48%)	8/17 (47%)	25/41 (61%)	16/38 (42%)
ICS+LTRA	8/11 (73%)	18/26 (69%)	15/28 (54%)	22/34 (65%)	59/81 (73%)	39/62 (63%)	1/5 (20%)	3/9 (33%)	1/0 (11%)	5/11 (46%)	16/27 (59%)	17/27 (63%)	2/5 (40%)	2/7 (29%)	10/12 (83%)
ICS+LABA+ LTRA	11/15 (73%)	23/37 (62%)	26/39 (67%)	13/16 (81%)	37/50 (74%)	25/33 (76%)	0/4 (0%)	3/21 (14%)	4/16 (25%)	14/21 (67%)	58/82 (71%)	47/66 (71%)	17/35 (49%)	58/101 (57%)	48/94 (51%)

Online Repository

METHODS

BREATHE study. This is cohort of children and young adults with doctor diagnosed asthma and was recruited from primary and secondary care in Tayside. Details of enrolment have been presented in detail previously[1]. Individuals aged >18 years were excluded from the present analysis.

GALA II study. The genes-environment and admixture in Latino Americans (GALA II) study is a multi-center study of children and young adults with and without asthma, and has been fully described previously[2]. Eligibility criteria were: aged 8-21 years of age, all four grandparents were Latino, <10 pack-years of smoking history. Asthma was defined based on physician diagnosis and report of symptoms and medication use within the last 2 years. Individuals aged >18 year and without asthma were excluded from this analysis. Estimates of African, European and Native American ancestries were obtained using an unsupervised analysis in ADMIXTURE[3] assuming 3 ancestral populations: African, European and Native American. We used reference haplotypes from individuals from HapMap phase II (http://hapmap.ncbi.nlm.nih.gov) for the European and African components: Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) and Yoruba in Ibadan, Nigeria (YRI). Native American reference population consisted of 71 Native American individuals genotyped at University of California San Francisco on the Axiom LAT1 array, including 14 Zapotec, 2 Mixe, and 11 Mixtec from the southern State of Oaxaca and 44 Nahua individuals from Central Mexico.

PAGES study. The Paediatric Asthma Gene Environment Study (PAGES, http://www.asthma-pages.com/) was designed to explore interactions between genetic variations and exposures (including medications) in children with asthma. Children were

recruited from primary and secondary care. Details of recruitment have been published earlier [4]. Ethnicity was not identified for the initial recruits to the study but was captured for approximately half participants and categorised as African, Chinese, Indian, Mixed, Pakistani, White British or other.

PACMAN cohort. The PACMAN study is a study of children aged 4-12 years recruited through community pharmacies in the Netherlands between 2009 and 2012. Details of the study protocol have been described elsewhere[5]. A detailed history of the subjects was obtained, including information on asthma symptoms, exacerbations and medication use over the preceding 12 months during a study visit in the community pharmacies. Ethnicity was categorised as African, Asian, Caucasian (Dutch, Turkish or Moroccan), Hispanic or Mixed.

PASS cohort. The Pharmaco genetics of Adrenal Suppression with inhaled Steroid study is a cohort study designed to explore the clinical and pharmacogenomics associations between use of corticosteroids in children with asthma and adrenal suppression. Assessment included a respiratory questionnaire, collection of blood or salvia for DNA extraction. Details of recruitment have been published previously[6]. Children with an Asian ancestry were excluded and ethnicity for those recruited was categorised as African, Caucasian or other.

Table I. The power of the sample size of the five cohorts combined to detect increases in odds ratio (OR) for asthma exacerbations

Treatment Group	OR=1.2	OR=1.5	OR=3
Short acting beta agonist alone	44%	96%	100%
Inhaled corticosteroid (ICS)	70%	99%	100%
ICS plus long acting beta agonist (LABA)	36%	91%	100%
ICS plus leukotriene receptor antagonist (LTRA)	20%	64%	100%
ICS plus LABA plus LTRA	34%	89%	100%

Table II. Odds ratio for exacerbation regardless of treatment in each cohort from the additive model. The odds ratio indicates the risk for each A allele of rs1042713. The logistic regression models adjusted for age, sex and exposure to cigarette smoke. The p value for the pooled analysis was 0.035.

	BREATHE	GALA II	PACMAN	PAGES	PASS	**POOLED**
Odds ratio [95% CI]	1.14 [0.97, 1.34] n=1210	1.18 [0.98, 1.41] n=1171	1.00 [0.69, 1.41] n=760	1.02 [0.82, 1.28] n=695	1.08 [0.81, 1.43] n=390	**1.11 [1.01,1.22]**

Table III. Odds ratio (OR) and 95% confidence interval [CI] for exacerbation for each Arg16 allele compared to Gly 16 homozygotes among children in receipt of daily short acting beta agonist (SABA) use regardless of other treatment. The logistic regression models adjusted for sex, age and exposure to cigarette smoke. The p value for the pooled analysis was 0.91.

	BREATHE	PACMAN	PAGES	**POOLED**
OR [95% CI] for children in receipt of SABA every day	1.13 [0.77, 1.65] n=250	1.41 [0.83, 2.40] n=364	0.69 [0.44, 1.10] n=208	**1.01 [0.79, 1.31]**

Table IV. Odds ratio (OR) and 95% confidence interval [CI] for exacerbation using the additive model (i.e. risk for each Arg16 allele compared to none) among children treated with inhaled corticosteroids and long acting beta agonists for the three populations where short acting beta agonist (SABA) use was determined. The logistic regression models adjusted for sex, age and exposure to cigarette smoke. The p values for the pooled analysis were 0.22 and 0.038.

	BREATHE	PACMAN	PAGES	**POOLED**
OR [95% CI] for children in receipt of SABA every day	1.02 [0.37, 2.81] n=44	4.57 [1.40, 14.89] n=74	0.89 [0.30, 2.63] n=47	**1.49 [0.79, 2.79]**
OR [95% CI] for children in receipt of SABA less than once a day	1.71 [0.94, 3.12] n=94	0.65 [0.06, 19.51] n=72	1.55 [0.80, 2.99] n=87	**1.59 [1.03, 2.45]**

REFERENCES

(1) Palmer CN, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta2 adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax 2006;61:940-944.

(2) Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, Thyne S, Farber HJ, Serebrisky D, Kumar R, Brigino-Buenaventura E, Davis A, LeNoir MA, Meade K, Rodriguez-Cintron W, Avila PC, Borrell LN, Bibbins-Domingo K, Rodriguez-Santana JR, Sen S, Lurmann F, Balmes JR, Burchard EG. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;188:309-318.

(3) Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 2009; 19:1655-64.

(4) Turner SW, Ayres JG, Macfarlane TV, Mehta A, Mehta G, Palmer CN, Cunningham S, Adams T, Aniruddhan K, Bell C, Corrigan D, Cunningham J, Duncan A, Hunt G, Leece R, MacFadyen U, McCormick J, McLeish S, Mitra A, Miller D, Waxman E, Webb A, Wojcik S, Mukhopadhyay S, Macgregor D. A methodology to establish a database to study gene environment interactions for childhood asthma. BMC Medical Research Methodology 2010;10:107.

(5) Koster ES, Raaijmakers JA, Koppelman GH, Postma DS, van der Ent CK, Koenderman L, Bracke M, Maitland-van der Zee AH. Pharmacogenetics of anti-inflammatory treatment in children with asthma: rationale and design of the PACMAN cohort. Pharmacogenomics 2009;10:1351-1361.

(6) Hawcutt DB, Jorgensen AL, Wallin N, Thompson B, Peak M, Lacy D, Newland P, Didi M, Couriel J, Blair J, Pirmohamed M, Smyth R. Adrenal Responses to a Low Dose Short Synacthen Test in Children with Asthma. *Clin Endocrinol* 2014;DOI: 10.1111/cen.12655.